

**WHAT IS CLAIMED IS:**

1. A flow-through assay device for detecting the presence or quantity of an analyte residing in a test sample, said flow-through assay device comprising a porous membrane, said porous membrane being in communication with detection probes and calibration probes, said detection probes being conjugated with a specific binding member for the analyte, said porous membrane defining:

a detection zone within which is immobilized a first capture reagent, said first capture reagent being configured to bind to at least a portion of said conjugated detection probes or complexes thereof to generate a detection signal having an intensity;

a compensation zone located downstream from said detection zone, wherein a second capture reagent is immobilized within said compensation zone, said second capture reagent being configured to bind to said conjugated detection probes or complexes thereof passing through said detection zone to generate a compensation signal having an intensity, wherein the intensity of said compensation signal is inversely proportional to the intensity of said detection signal; and

a calibration zone within which a third capture reagent is immobilized, said third capture reagent being configured to bind to said calibration probes to generate a calibration signal that is substantially constant in intensity relative to the intensities of said detection signal and said compensation signal;

wherein the amount of the analyte within the test sample is proportional to the ratio of said detection signal intensity to said compensation signal intensity, as calibrated by said calibration signal intensity.

2. A flow-through assay device as defined in claim 1, wherein said conjugated detection probes comprise a substance selected from the group consisting of chromogens, catalysts, luminescent compounds, radioactive compounds, visual labels, liposomes, and combinations thereof.

3. A flow-through assay device as defined in claim 1, wherein said conjugated detection probes comprise a luminescent compound.

4. A flow-through assay device as defined in claim 1, wherein said conjugated detection probes comprise a visual label.

5. A flow-through assay device as defined in claim 1, wherein said specific

binding member is selected from the group consisting of antigens, haptens, aptamers, primary or secondary antibodies, biotin, and combinations thereof.

5 6. A flow-through assay device as defined in claim 1, wherein said first capture reagent is selected from the group consisting of antigens, haptens, protein A or G, neutravidin, avidin, streptavidin, captavidin, primary or secondary antibodies, and complexes thereof.

10 7. A flow-through assay device as defined in claim 1, wherein said second capture reagent is selected from the group consisting of antigens, haptens, protein A or G, neutravidin, avidin, streptavidin, captavidin, primary or secondary antibodies, and complexes thereof.

8. A flow-through assay device as defined in claim 1, wherein said second capture reagent comprises a polyelectrolyte.

15 9. A flow-through assay device as defined in claim 8, wherein said polyelectrolyte has a net positive charge.

20 10. A flow-through assay device as defined in claim 9, wherein said polyelectrolyte is selected from the group consisting of polylysine, polyethyleneimine, epichlorohydrin-functionalized polyamines or polyamidoamines, polydiallyldimethyl-ammonium chloride, cationic cellulose derivatives, and combinations thereof.

25 11. A flow-through assay device as defined in claim 8, wherein said polyelectrolyte has a net negative charge.

30 12. A flow-through assay device as defined in claim 1, wherein said third capture reagent comprises antigens, haptens, protein A or G, neutravidin, avidin, streptavidin, captavidin, primary or secondary antibodies, or complexes thereof.

13. A flow-through assay device as defined in claim 1, wherein the device is a sandwich-type assay device.

14. A flow-through assay device for detecting the presence or quantity of an analyte residing in a test sample, said flow-through assay device comprising a porous membrane, said porous membrane being in communication with optical detection probes and optical calibration probes, said optical detection probes being conjugated with a specific binding member for the analyte, said porous membrane defining:

a detection zone within which a first capture reagent is immobilized, said

first capture reagent being configured to bind to at least a portion of complexes formed between the analyte and said conjugated optical detection probes to generate a detection signal intensity;

5 a compensation zone located downstream from said detection zone, wherein a second capture reagent is immobilized within said compensation zone, said second capture reagent being configured to bind to said conjugated optical detection probes and said complexes passing through said detection zone to generate a compensation signal intensity, wherein the intensity of said compensation signal is inversely proportional to the intensity of said detection  
10 signal; and

a calibration zone within which a third capture reagent is immobilized, said third capture reagent being configured to bind to said optical calibration probes to generate a calibration signal intensity that is substantially constant relative to the intensities of said detection signal and said calibration signal;

15 wherein the amount of the analyte within the test sample is proportional to the ratio of said detection signal intensity to said compensation signal intensity, as calibrated by said calibration signal intensity.

15. A flow-through assay device as defined in claim 14, wherein said conjugated optical detection probes comprise a luminescent compound.

20 16. A flow-through assay device as defined in claim 14, wherein said conjugated optical detection probes comprise a visual label.

17. A flow-through assay device as defined in claim 14, wherein said first capture reagent is selected from the group consisting of antigens, haptens, protein A or G, neutravidin, avidin, streptavidin, captavidin, primary or secondary  
25 antibodies, and complexes thereof.

18. A flow-through assay device as defined in claim 14, wherein said second capture reagent is selected from the group consisting of antigens, haptens, protein A or G, neutravidin, avidin, streptavidin, captavidin, primary or secondary antibodies, and complexes thereof.

30 19. A flow-through assay device as defined in claim 14, wherein said second capture reagent comprises a polyelectrolyte.

20. A flow-through assay device as defined in claim 14, wherein said third capture reagent comprises antigens, haptens, protein A or G, neutravidin, avidin,

streptavidin, captavidin, primary or secondary antibodies, or complexes thereof.

21. A method for detecting the presence or quantity of an analyte residing in a test sample, said method comprising:

5 i) providing a flow-through assay device comprising a porous membrane, said porous membrane being in communication with detection probes and calibration probes, said detection probes being conjugated with a specific binding member for the analyte, said porous membrane defining a detection zone within which a first capture reagent is immobilized, a compensation zone within which a second capture reagent is immobilized, and a calibration zone within which a third capture reagent is immobilized, wherein said compensation zone is located downstream from said detection zone;

10 ii) contacting a test sample containing the analyte with said conjugated detection probes and said calibration probes;

15 iii) measuring a detection signal intensity generated at said detection zone, a compensation signal intensity generated at said compensation zone, and a calibration signal intensity generated at said calibration zone;

iv) comparing the intensity of said detection signal to said compensation signal, wherein the intensity of said compensation signal is inversely proportional to the intensity of said detection signal; and

20 v) calibrating the compared intensities of said detection signal and said compensation signal with the intensity of said calibration signal, wherein the intensity of said calibration signal is substantially constant relative to the intensities of said detection signal and said calibration signal.

22. A method as defined in claim 21, wherein said conjugated detection probes comprise a substance selected from the group consisting of chromogens, catalysts, luminescent compounds, radioactive compounds, visual labels, liposomes, and combinations thereof.

23. A method as defined in claim 21, wherein said conjugated detection probes comprise a luminescent compound.

24. A method as defined in claim 21, wherein said conjugated detection probes comprise a visual label.

25. A method as defined in claim 21, wherein said specific binding member is selected from the group consisting of antigens, haptens, aptamers, primary or

secondary antibodies, biotin, and combinations thereof.

26. A method as defined in claim 21, wherein said first capture reagent is selected from the group consisting of antigens, haptens, protein A or G, neutravidin, avidin, streptavidin, captavidin, primary or secondary antibodies, and complexes thereof.

27. A method as defined in claim 21, wherein said second capture reagent is selected from the group consisting of antigens, haptens, protein A or G, neutravidin, avidin, streptavidin, captavidin, primary or secondary antibodies, and complexes thereof.

28. A method as defined in claim 21, wherein said second capture reagent comprises a polyelectrolyte.

29. A method as defined in claim 21, further comprising generating a calibration curve by plotting the ratio of said detection signal to said compensation signal calibrated by the intensity of said calibration signal for a plurality of predetermined analyte concentrations.

30. A method for detecting the presence or quantity of an analyte residing in a test sample, said method comprising:

i) providing a flow-through assay device comprising a porous membrane, said porous membrane being in communication with optical detection probes and optical calibration probes, said optical detection probes being conjugated with a specific binding member for the analyte, said porous membrane defining a detection zone within which a first capture reagent is immobilized, a compensation zone within which a second capture reagent is immobilized, and a calibration zone within which a third capture reagent is immobilized, wherein said compensation zone is located downstream from said detection zone;

ii) contacting a test sample containing the analyte with said optical conjugated detection probes and said optical calibration probes;

iii) optically measuring a detection signal intensity generated at said detection zone, a compensation signal intensity generated at said compensation zone, and a calibration signal intensity generated at said calibration zone;

iv) comparing the intensity of said detection signal to said compensation signal, wherein the intensity of said compensation signal is inversely proportional to the intensity of said detection signal; and

v) calibrating the compared intensities of said detection signal and said compensation signal with the intensity of said calibration signal, wherein the intensity of said calibration signal is substantially constant relative to the intensities of said detection signal and said calibration signal.

5           31. A method as defined in claim 30, wherein said optical detection probes comprise a luminescent compound.

          32. A method as defined in claim 30, wherein said optical detection probes comprise a visual label.

10           33. A method as defined in claim 30, wherein said specific binding member is selected from the group consisting of antigens, haptens, aptamers, primary or secondary antibodies, biotin, and combinations thereof.

          34. A method as defined in claim 30, wherein said first capture reagent is selected from the group consisting of antigens, haptens, protein A or G, neutravidin, avidin, streptavidin, captavidin, primary or secondary antibodies, and  
15           complexes thereof.

          35. A method as defined in claim 30, wherein said second capture reagent is selected from the group consisting of antigens, haptens, protein A or G, neutravidin, avidin, streptavidin, captavidin, primary or secondary antibodies, and  
20           complexes thereof.

          36. A method as defined in claim 30, wherein said second capture reagent comprises a polyelectrolyte.

          37. A method as defined in claim 30, further comprising generating a calibration curve by plotting the ratio of said detection signal to said compensation signal calibrated by the intensity of said calibration signal for a plurality of  
25           predetermined analyte concentrations.

          38. A method as defined in claim 30, further comprising exciting said optical conjugated detection probes at said detection zone and said compensation zone, wherein said excitation causes said optical conjugated detection probes to emit  
30           said detection signal and said compensation signal.